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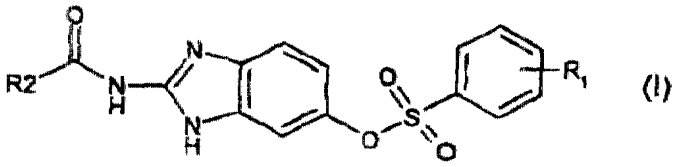
(54) Substituted benzimidazole compounds and their use for the treatment of cancer

(57) New benzimidazole compounds of formula (I) and their use as cyclin-dependent kinases inhibitors compounds as well as new pharmaceutical compositions containing them.



(I)

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Description

Technical Field

[0001] The present invention relates to compounds useful for treating pathological states, which arise from or are exacerbated by cell proliferation, to pharmaceutical compositions comprising these compounds, and to methods of inhibiting cell proliferation in a mammal.

Background of the Invention

[0002] Neoplastic diseases, characterized by the proliferation of cells, which are not subject to normal cell proliferating controls, are a major cause of death in humans and other mammals. Cancer chemotherapy has provided new and more effective drugs to treat these diseases and has also demonstrated that drugs, which are inhibitors of cyclin-dependent kinases are effective in inhibiting the proliferation of neoplastic cells.

[0003] Regulators at cell cycle checkpoints determine the decision for a cell to proceed through the cell cycle. Progression of the cell cycle is driven by cyclin-dependent kinases (CDKs) which are activated by oscillating members of the cyclin family, resulting in substrats phosphorylation and ultimately cell division. In addition, endogenous inhibitors of CDKs (INK4 family and KIP/CIP family) negatively regulate the activity of CDKs. Normal cell growth is due to a balance between activators of CDKs (cyclins) and endogenous inhibitors of CDKS. In several types of cancer, aberrant expression or activity of several components of the cell cycle has been described.

[0004] Cdk4 fonctions in GI phase of the cell cycle and is activated by D-type cyclins, which results in substrate phosphorylation and progression to S phase. The only known substrate for cdk4 is the retinoblastoma gene product (pRb), a major tumor suppressor gene product, which fonctions as a major checkpoint control in regulation of the GI/S phase transition. Hyperphosphorylation of pRb by CDKs causes the release of E2F (a family of transcription factors) bound to pRb which then activate genes necessary for cell cycle progression, e.g. thymidine kinase, thymidylate synthase, cyclin E and cyclin A. Cyclin DI is amplified or overexpressed in many types of cancer (breast, ovarian, bladder, esophogeal, lung, lymphoma), while the gene for p16, the endogenous inhibitor of cdk4, is deleted, mutated, or aberrantly methylated in many tumor types. A point mutation in cdk4 was reported in a melanoma tumor that rendered the enzyme unable to bind p1 6 resulting in a constitutively active enzyme. All of the conditions described above lead to activation of cdk4 and cell cycle progression and tumor cell growth.

[0005] An added level of regulation of CDK activity exists. Cyclin-dependent kinase activating kinase (CAK) is a positive regulator of CDKs. CAK phosphorylates the catalytic CDKs on a conserved threonine residue to render the target enzyme completely active.

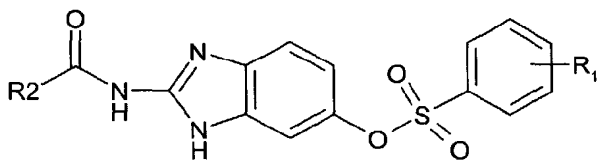
[0006] Because the defects in cell cycle molecules lead to CDK activation and subsequently cell cycle progression, it is logical that inhibition of CDK enzyme activity should block cell cycle progression and tumor cell growth.

[0007] The first CDK inhibitor to enter clinical trials is the compound known as Flavopiridol. This compound is currently in Phase II clinical trials and is the only molecule in its class in the clinic at the present time. The aim of this invention is to produce molecules more active that Flavopiridol.

[0008] It is known following publication of WO00/41669 that benzimidazole carbamate derivatives are vascular damaging agents that can be used for treating cancer, the sulfonoester derivatives claimed in this patent application are not at all exemplified and their anticancerous way of action is not described. Our invention relates speciffically to sulfonesters derivatives of those carbamates.

Summary of the Invention

[0009] In one embodiment of the present invention are disclosed compounds of formula (I)



or a pharmaceutically acceptable salt, ^

- wherein R_1 is selected from the group consisting of
  - $4NH_2$
  - 4 NH alkyl or cycloalkyl eventually substituted with an an acyl group, an hydroxy, an amino, alkoxy, heterocyclyl or aryl group
  - 4 N imidazolyl
  - 3 SO_2 Me
- wherein R2 is selected from the group consisting of
  - alkyl eventually substituted by amino, acid, acid derivative, alkoxy, aryl or OH groups
  - arylalkyl eventually substituted by alkoxy, halogeno, amino, acid or acid derivatives
  - alkoxy eventually substituted by aryl
  - amino, NHR_3, NR_3R_4 wherein R_3 and R_4 are selected independtly from hydrogen, alkyl, alkylaryl, aryl or together form an alkylen chain.

[0010] Among the aryl substituents are included the aryl groups substituted with one or more groups selected from halogen, alkoxy, alkyl, amino, nitro.

[0011] Among the alkyl substituents are included the alkyl groups substituted with one or more amino, acyl, acyl derivatives, aminoacyl derivatives, alkoxy, arylalkyl or aryl groups.

[0012] Among the alkoxy substituents are included the alkoxy groups substituted with one or more amino, acyl, acyl derivatives, alkyl, arylalkyl or aryl groups

[0013] Among the acyl groups or acyl derivatives groups are included the carboxylic acids and the sulfonic acids, the derivatives of which being mainly ester derivatives.

[0014] The alkyl chain of the present invention includes linear, ramified or cyclic chain containing 1 to 7 carbon atoms. The alkoxy chain of the present invention includes linear, ramified or cyclic chain containing 1 to 4 carbon atoms. The aryl groups includes phenyl group, heterocyclyl groups containing one or two heteroatoms choosen from S, N or O such as furyl or pyridine.

[0015] Among those compounds are preferred those containing in the alkyl chain 1 to 4 carbon atoms and those containing in the cycloalkyl chain 3 to 5 carbon atoms. When the alkyl chain is substituted by an alkoxy group this last group has preferably one carbon atom.

[0016] In the compounds of formula (I) are preferred those containing for R_2 an amino substituent and preferably a monoalkylamino or a monoarylamino substituent and still more preferably those containing a monoalkylamino substituent with an acylderivative..

[0017] Among the compounds of formula (I) the following compounds are much more preferred:

Methyl-5-(4-[2-hydroxyethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[4-hydroxbutyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[2-methoxyethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[1-imidazolyl]-phenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[2-pyridylmethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-ethylaminophenylsulfonyloxy) benzimidazole-2-carbamateMethyl-5-(4-[N-Glycinyl]-phenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[1-methyl,2-hydroxyethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[2-methyl,2-hydroxyethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-isopropylaminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[1-ethyl, 2-hydroxyethyl]aminophenyl sulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-butylaminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[3-methoxypropyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-methylaminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[2-sulfonylethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-aminophenylsulfonyloxy) benzimidazole-2-carbamate

Methyl-5-(4- [2-diethylaminoethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[1-tetrathydrofurylmethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-cyclopentylaminophenylsulfonyloxy) benzimidazole-2-carbamate
Methyl-5-(4-[2-phenylethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate
N-[5-(4-[1-imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]succinamic acid-methyl-ester
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl] *tert*-butoxycarbonyl glycine amide
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-succinamic acid methyl-ester
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-butyric acid methyl-ester
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-cyclopropanecarbonylamide
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-methoxyaceticamide
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-dimethylamino-acetylamide
N-[5-(4-[imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]-methylurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-methylurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-dimethylurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-cyclopropylurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-isopropylamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-butylamineurea
N-[5-(4-[imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]2-fluoro-anilineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- I H-benzimidazole-2-yl]-2-fluoro-anilineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-*m*-anisidineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-*p*-anisidineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-4-chloroanilineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-3-fluoro-anilineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-3-chloroanilineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-isobutylamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-N,N-dimethylethylenediamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-ethylamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-glycineurea N-[5-(4-[imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]-2-aminoethanesulfonic acidurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-2-methoxyethylamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-N,N-dimethyl-1,4-phenylenediamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-2-aminomethylpyridineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-cyclobutylamineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-4-(aminomethyl)pyridineurea
N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]- *tert*-butylamineurea

[0018] In still yet another embodiment is disclosed use of compounds of formula (I) for treating cancer diseases.

[0019] In still yet another embodiment is disclosed a method of inhibiting CDK4 enzymes in a mammal in recognized need of such treatment comprising administering to the mammal a therapeutically effective amount of compounds of formula (I).

[0020] In still yet another embodiment is disclosed a pharmaceutical composition which comprises a therapeutically effective amount of a compound of formula (I) in combination with a pharmaceutically acceptable carrier.

[0021] The term "pharmaceutically acceptable salt", as used herein, refers to salts, which are suitable for use in contact with the tissues of humans and lower animals. Pharmaceutically acceptable salts are described in detail in J. Pharmaceutical Sciences, 1977, 66:1 et seq. hereby incorporated by reference. Representative acid addition salts include acetate, citrate, aspartate, benzenesulfonate, hydrochloride, lactate, maleate, methanesulfonate, oxalate, and phosphate.

Methods of Synthesis

[0022] Compounds of the present invention can be easily prepared starting from 2-amino-5-(-4-fluorophenylsulfonyloxy)nitrobenzene the process of preparation of which is described in US 3,996,368.

[0023] In a first step this starting material is reacted with the amine bearing the R1 radical in a suitable solvent for carrying out the reaction. Among the list of solvents suitable for dissolving 2-amino-5-(-4-fluorophenylsulfonyloxy)nitrobenzene and the amine can be cited the glycols such as ethylglycol, the aprotic solvents such as dioxane, dimethylformamide, N-methylpyrrolidone. The preferred temperature for this reaction is comprised between room temperature and the reflux temperature. To recover the intermediate product it is preferred to precipitate the intermediate with chlorhydric acid.

[0024] In a second step the compound of step 1 is hydrogenated with hydrogen preferably in presence of Raney nickel (nitro group reduction method A) or palladium on carbon (nitro group reduction method B) in a suitable solvent choosen among the same list as for step 1 in mixture with an alcohol such as methanol. After reaction the catalyst is taken off by filtration.

[0025] In a third step the benzimidazole ring is closed by action of 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea on the intermediate obtained in step 2 without intermediate separation. The reaction mixture is heated to reflux with stirring. The final product (methyl-benzimidazole-2-carbamate) is isolated after evaporation of the solvent under reduced pressure and solubilization in ethylacetate then crystallisation. A final purification is carried out in methanol with a crystallisation in the same solvent.

[0026] Methyl-benzimidazole-2-carbamate can be converted to benzimidazole-2-ureas by treatment with an amine in a suitable solvent such as dimethylformamide, tetrahydrofuran or N-methylpyrrolidone in the presence of a base such as 1,8-Diazabicyclo[5.4.0]undec-7-ene in a pressure vessel.. The preferred temperature for this reaction is comprised between room temperature and 120 °C.

[0027] *tert*-butyl-benzimidazole-2-carbamate can be prepared by performing the third step described above using 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea instead of 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea. These derivatives can be converted to the corresponding 2-aminobenzimidazole derivative using *tert*-butylcarbamate deprotection methods known by the persons skilled in the art. The 2-aminobenzimidazoles can be converted to the corresponding amides by reaction with carboxylic acid derivatives using known by the persons skilled in the art.

Methods of Treatment

[0028] The present invention also provides pharmaceutical compositions, which comprise compounds of the present invention formulated together with one or more non-toxic pharmaceutically acceptable carriers. The pharmaceutical compositions may be specially formulated for oral administration in solid or liquid form or for parenteral injection.

[0029] The term "parenteral", as used herein, refers to modes of administration, which include intravenous, intramuscular, intraperitoneal, subcutaneous and infusion.

[0030] Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier.

[0031] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules.

[0032] The compounds of the present invention may be administered alone or mixed with other anticancer agents. Among the possible combinations, there may be mentioned

- alkylating agents and in particular cyclophosphamide, melphalan, ifosfamide, chlorambucil, busulfan, thiotepa, prednimustine, carmustine, lomustine, semustine, streptozotocin, decarbazine, temozolomide, procarbazine and hexamethylmelamine
- platinum derivatives such as in particular cisplatin, carboplatin or oxaliplatin,
- antibiotic agents such as in particular bleomycin, mitomycin, dactinomycin,
- antimicrotubule agents such as in particular vinblastine, vincristine, vindesine, vinorelbine, taxoids (paclitaxel and docetaxel),
- anthracyclines such as in particular doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone, losoxantrone,
- group I and II topoisomerases such as etoposide, teniposide, amsacrine, irinotecan, topotecan and tomudex,
- fluoropyrimidines such as 5-fluorouracil, UFT, floxuridine,
- cytidine analogues such as 5-azacytidine, cytarabine, gemcitabine, 6-mercaptomurine, 6-thioguanine,
- adenosine analogues such as pentostatin, cytarabine or fludarabine phosphate,
- methotrexate and folinic acid,
- various enzymes and compounds such as L-asparaginase, hydroxyurea, trans-retinoic acid, suramine, dexrazoxane, amifostine, herceptin as well as oestrogenic and androgenic hormones.

[0033] It is also possible to combine a radiation treatment with the compounds of the present invention. This treatment may be administered simultaneously, separately or sequentially. The treatment will be adapted to the patient to be treated by the practitioner.

[0034] The invention will be more fully described by the following examples, which must not be considered as a limitation of the invention.

Method for analytical determination

Liquid chromatography coupled to Mass spectrometry (LC/MS) analysis

[0035] LC/MS analyses were conducted on a Micromass instrument model LCT linked to an HP 1100 model instrument. Compound abundance were detected using an HP G1315A (model) photodiode array detector in the 200-600 nm wavelength range and a Sedex 65 (model) evaporative light scattering detector. Mass spectra were acquired in the 160 to 2000 amu range. Data were analysed using the Micromass MassLynx software. Separation were carried out on a Hypersil Highpurity C18, 5 μm particle size column (50 x 4.6 mm) eluted by a linear gradient of 10 to 90 % acetonitrile containing 0.05 % (v/v) trifluoroacetic acid (TFA) in water containing 0.05 % (v/v) TFA in 6.50 min at a flow rate of 1 ml/min.

Method for Purification

LC/MS triggered purification

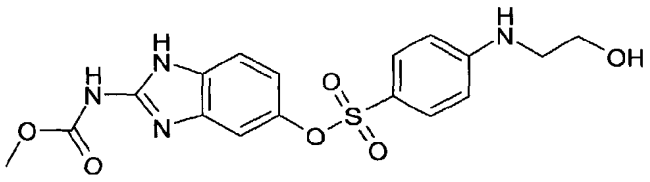
[0036] Compounds were purified by LC/MS using a Waters FractionLynx system composed of a Waters model 600 gradient pump, a Waters model 515 regeneration pump, a Waters Reagent Manager make-up pump, a Waters model 2700 sample manager autoinjector, two Rheodyne model LabPro switches, a Waters model 996 photodiode array detector, a Waters model ZMD mass spectrometer and a Gilson model 204 fraction collector. The Waters FractionLynx software controlled the instrument. Separation were conducted alternatively on two Waters Symmetry columns (C_{18}; 5 μM, 19 x 50 mm, catalogue number 186000210), one column was under regeneration by a 95/5 (v/v) water/acetonitrile mixture containing 0.07 % TFA (v/v) while the other one is separating. Columns were eluted by a linear gradient of acetonitrile containing 0.07 % (v/v) TFA in water containing 0.07 % (v/v) TFA, from 5 to 95 % (v/v) in 8 min and 2 min at 95 % acetonitrile containing 0.07 % (v/v) TFA, at a flow rate of 10 ml/min. At the output of the separating column the flow was split to the 1/1000 ratio using a LC Packing AccuRate splitter; 1/1000 of the flow was mixed with methanol (0.5 ml/min. flow rate) and sent to the detectors, this flow was split again ¾ of the flow was sent to the photodiode array detector and ¼ to the mass spectrometer; the rest of the output of the column (999/1000) was sent to the fraction collector were flow was directed normally to waste unless expected mass signal was detected by the FractionLynx software. The FractionLynx software was supplied with molecular formulas of expected compounds and triggered the collection of compounds when mass signal corresponding to $[M+H]^+$ and $[M+Na]^+$ are detected. In certain cases (depending on analytical LC/MS result, when $[M+2H]^{++}$ was detected as an intense ion) the FractionLynx software was additionally supplied with calculated half molecular weight (MW/2), in these conditions collection was also triggered when mass signal corresponding to $[M+2H]^{++}$ and $[M+Na+H]^{++}$ are detected. Compounds were collected in tarred glass tubes. After collection, solvent was evaporated in a Jouan model RC 10.10 centrifuge evaporator or a Genevac model HT8 centrifuge evaporator and the amount of compound was determined by weighing of the tubes after solvent evaporation.

Method of prepartion of compounds of the invention

[0037] 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene (melting point 161°C), the starting material, can be prepared according to U.S. patent N° 3,996,368.

Example 1 : Preparation of Methyl-5-(4-[2-hydroxyethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate

[0038]



step 1 : 15.6 g of 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene were combined with 25 ml ethanolamine in 100ml ethylglycol in a round bottom flask. The reaction mixture was heated to reflux for 90 min and then cooled on ice. Reaction mixture was then diluted with 250 ml of 2N aqueous HCl, the compound precipitated and was

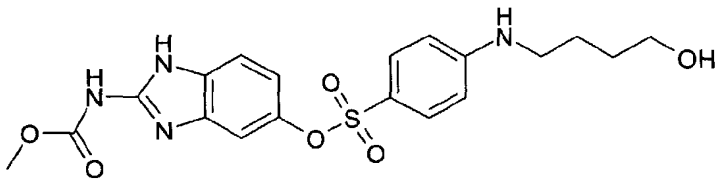
filtered off with suction. The preciptate was the washed with water and dried, yielding 15.5g of 2-amino-5-(4-[2-hydroxyethyl] aminophenylsulfonyloxy)nitro benzene (melting point 180°C)

step 2: 15.5 g of 2-amino-5-(4-[2-hydroxyethyl] aminophenylsulfonyloxy)nitro-benzene in 75 ml of methanol and 75 ml of dimethylformamide are hydrogenated under atmospheric pressure with a catalytic amount of Raney Nickel (method A). After hydrogen uptake is complete, the catalyst was filtered off with suction, washed with methanol and the filtrate is concentred under reduced pressure

step 3 : concentrated filtrate of step 2 was taken up in 150 ml methanol and 30 ml of glacial acetic acid, 10.3 g of 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea was added and reaction mixture was heated to reflux with stirring for 3 hours. Solvents were then evaporated under reduced pressure, concentrate was then dissolved in hot ethylacetate, crystallized by cooling and washed with ethylacetate. Compound was then solubilized in 250 ml refluxing methanol, crystallized by cooling and washed with methanol and dried yielding 7.4 g of the title compound. (Melting point 170°C, LC/MS analysis: retention time = 2.8 min., mass spectrum: 407.24, $[M+H]^+$)

Example 2 : Preparation of Methyl-5-(4-[4-hydroxbutyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate

[0039]



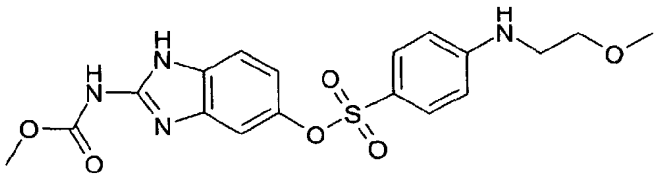
step 1 : 19.7 g of 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene were combined with 20 g butanolamine in 200 ml N-methylpyrrolidinone in a round bottom flask. The reaction mixture was heated to reflux for 120 min and then solvent was evaporated under reduced pressure. Concentrate was then solubilized with ethylacetate and extracted with 2N aqueous HCl and water and then dried over sodium sulfate and dried under reduced pressure. The concentrate was recrystallized in isopropanol, filtered under suction, washed with isopropanol and dried, yielding 13.1 g of 2-amino-5-(4-[4-hydroxbuyl] aminophenylsulfonyloxy)nitrobenzene (melting point 105°C).

step 2 : 13.1 g of 2-amino-5-(4-[4-hydroxbutyl] aminophenylsulfonyloxy)nitro-benzene in 75 ml of methanol and 75 ml of dimethylformamide are hydrogenated under atmospheric pressure with a catalytic amount of Raney Nickel (Method A). After hydrogen uptake is complete, the catalyst was filtered off with suction, washed with methanol and the filtrate is concentred under reduced pressure.

step 3 : concentrated filtrate of step 2 was taken up in 100 ml methanol and 20 ml of glacial acetic acid, 8.2 g of 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea was added and reaction mixture was heated to reflux with stirring for 3 hours. Solvents were then evaporated under reduced pressure, concentrate washed with 2N aqueous ammonia, water and dried. Concentrate was then dissolved in hot ethylacetate, crystallized by cooling and washed with ethylacetate. Compound was then solubilized in refluxing methanol, crystallized by cooling and washed with methanol and dried yielding 6.3 g of the title compound. (Melting point 180°C, LC/MS analysis: retention time = 2.9 min., mass spectrum: 435.29, $[M+H]^+$)

Example 3 : Preparation of Methyl-5-(4-[2-methoxyethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate

[0040]



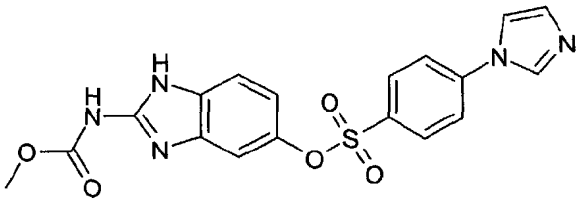
step 1 : 15.6 g of 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene were combined with 35 ml methoxyethylamine in 100ml dioxane in a round bottom flask. The reaction mixture was heated to reflux for 8 hours and then cooled to 40°C and extracted two times with 250 ml water. Concentrate was solubilized with ethylacetate and extracted with 2N aqueous HCl and water, the organic phase was then dried under reduced pressure, yielding 19.2 g of 2-amino-5-(4-[2-methoxyethyl] aminophenylsulfonyloxy)nitrobenzene (melting point 105°C).

step 2 : 18.2 g of 2-amino-5-(4-[2-methoxyethyl] aminophenylsulfonyloxy)nitro-benzene in 75 ml of methanol and 75 ml of dimethylformamide are hydrogenated under atmospheric pressure with a catalytic amount of Raney Nickel (Method A). After hydrogen uptake is complete, the catalyst was filtered off with suction, washed with methanol and the filtrate is concentred under reduced pressure.

step 3 : concentrated filtrate of step 2 was taken up in 150 ml methanol and 25 ml of glacial acetic acid, 12.3 g of 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea was added and reaction mixture was heated to reflux with stirring for 3 hours. Solvents were then evaporated under reduced pressure, and concentrate was crystallized with methanol saturated with ammonia, washed with water, methanol and dried, yielding 12 g of the title compound. (Melting point 155°C, LC/MS analysis: retention time = 3.1 min., mass spectrum: 421.25, $[M+H]^+$)

Example 4 : Preparation of Methyl-5-(4-[1-imidazolyl]-phenylsulfonyloxy) benzimidazole-2-carbamate

[0041]



step 1 : 15.6 g of 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene were combined with 20.7 g imidazole in 100 ml dimethylformamide in a round bottom flask. The reaction mixture was heated to reflux for 3 hours and then cooled to room temperature. Reaction mixture was then precipitated by addition of water filtered and precipitate was washed with water and dried. Residue was resolubilized in hot methylglycol, crystallized by cooling and the crystals were washed with methanol and dried, yielding 10.4 g of 2-amino-5-(4-[1-imidazolyl]-phenylsulfonyloxy) nitro-benzene (melting point 209°C).

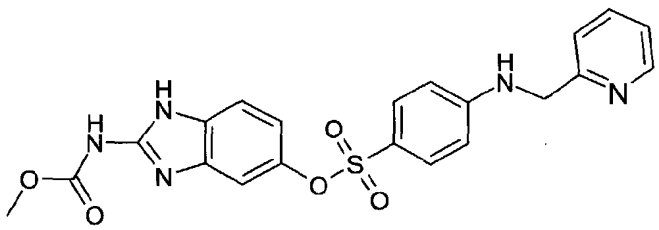
step 2 : 10.4 g of 2-amino-5-(4-[1-imidazolyl]-phenylsulfonyloxy)nitrobenzene in 75 ml of methanol and 75 ml of dimethylformamide are hydrogenated under atmospheric pressure with a catalytic amount of Raney Nickel. After hydrogen uptake is complete, the catalyst was filtered off with suction, washed with methanol and the filtrate is concentred under reduced pressure (Method A).
Alternatively 5 g of 2-amino-5-(4-[1-imidazolyl]-phenylsulfonyloxy)nitrobenzene in 475 ml of methanol and 25 ml of dimethylformamide are hydrogenated under 5 bars pressure with 10 % (w/w) of palladium on carbon at 30°C during 6 hours (Method B) yielding 4.18 g (91 %) of expected product.

step 3: concentrated filtrate of step 2 was taken up in 150 ml methanol and 25 ml of glacial acetic acid, 10.3 g of

1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea was added and reaction mixture was heated to reflux with stirring for 3 hours. After cooling to room temperature reaction mixture was precipitated by addition of ethylacetate, filtered by suction and washed by ethylacetate. Filtrate was then resolubilized with 50 ml dimethylformamide and 250 ml of methanol was added. Mixture crystallised upon cooling and crystals were washed with methanol and dried under reduced pressure, yielding 9.4 g of the title compound. (Melting point 258°C, LC/MS analysis: retention time = 2.5 min., mass spectrum: 414.23, $[M+H]^+$; 382.19 fragmentation of carbamate: loss of methanol, NMR, IR).

Example 5 : Preparation of Methyl-5-(4-[2-pyridylmethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate

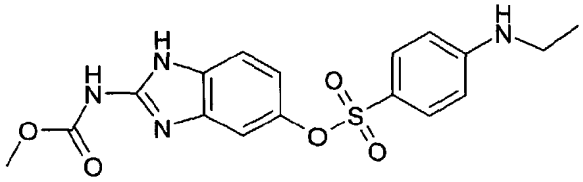
[0042]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 2-aminomethylpyridine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.6 min., mass spectrum: 454.28, $[M+H]^+$; 907.53, $[2M+H]^+$; 422.24 , fragmentation of carbamate: loss of methanol).

Example 6 : Preparation of Methyl-5-(4-ethylaminophenylsulfonyloxy) benzimidazole-2-carbamate

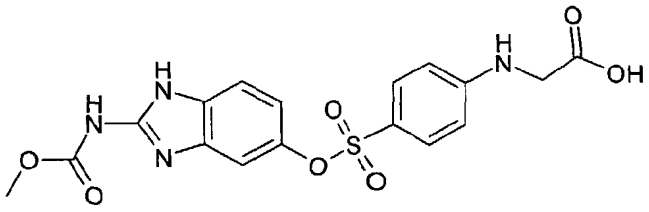
[0043]



In a similar manner to examples 1 to 4, title compound was obtained by reacting ethylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.2 min., mass spectrum: 390.98, $[M+H]^+$).

Example 7 : Preparation of Methyl-5-(4-[N-Glycinyl]-phenylsulfonyloxy) benzimidazole-2-carbamate

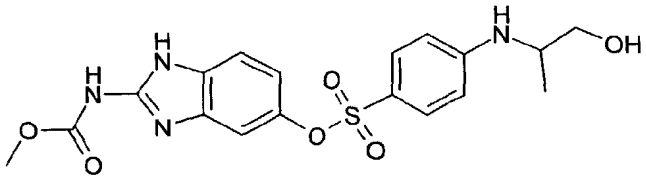
[0044]



In a similar manner to examples 1 to 4, title compound was obtained by reacting glycine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.8 min., mass spectrum: 421.21, $[M+H]^+$).

Example 8 : Preparation of Methyl-5-(4-[1-methyl,2-hydroxyethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate

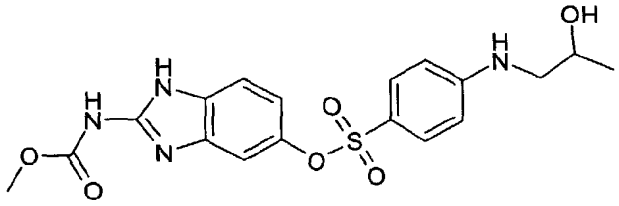
[0045]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 2-aminopropanol with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.9 min., mass spectrum: 421.27, $[M+H]^+$).

Example 9 : Preparation of Methyl-5-(4-[2-methyl,2-hydroxyethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate

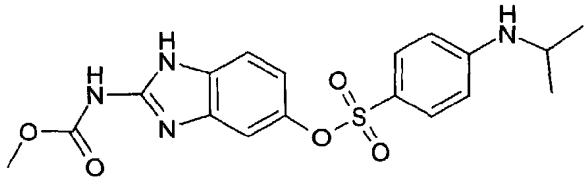
[0046]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 1-methyl, 2-aminoethanol with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.9 min., mass spectrum: 421.27, $[M+H]^+$).

Example 10: Preparation of Methyl-5-(4-isopropylaminophenylsulfonyloxy) benzimidazole-2-carbamate

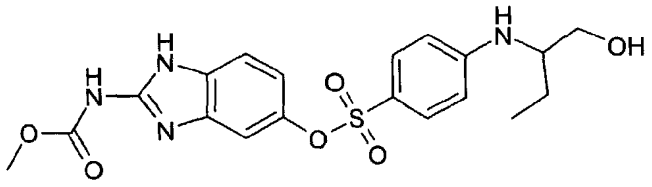
[0047]



In a similar manner to examples 1 to 4, title compound was obtained by reacting isopropylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.4 min., mass spectrum: 405.27, $[M+H]^+$).

Example 11 : Preparation of Methyl-5-(4-[1-ethyl, 2-hydroxyethyl]aminophenyl sulfonyloxy) benzimidazole-2-carbamate

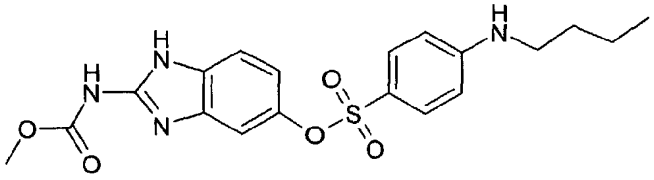
[0048]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 2-aminobutanol with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.0 min., mass spectrum: 435.30, $[M+H]^+$).

Example 12 : Preparation of Methyl-5-(4-butylaminophenylsulfonyloxy) benzimidazole-2-carbamate

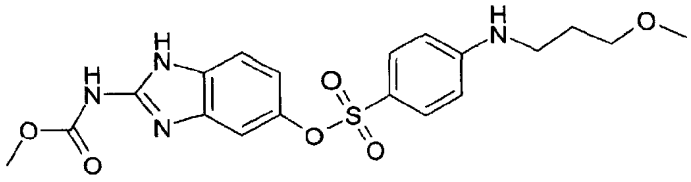
[0049]



In a similar manner to examples 1 to 4, title compound was obtained by reacting butylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.6 min., mass spectrum: 419.25, $[M+H]^+$).

Example 13 : Preparation of Methyl-5-(4-[3-methoxypropyl]aminophenyl-sulfonyloxy) benzimidazole-2-carbamate

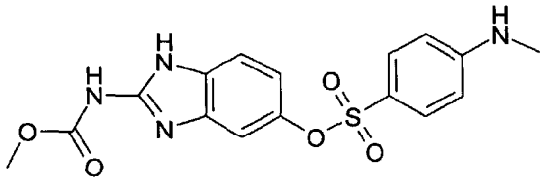
[0050]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 3-methoxypropanolamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.2 min., mass spectrum: 435.27, $[M+H]^+$).

Example 14 : Preparation of Methyl-5-(4-methylaminophenylsulfonyloxy) benzimidazole-2-carbamate

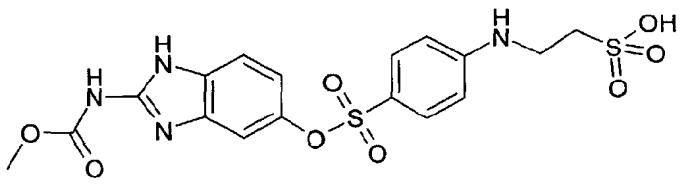
[0051]



In a similar manner to examples 1 to 4, title compound was obtained by reacting methylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.0 min., mass spectrum: 377.22, $[M+H]^+$).

Example 15 : Preparation of Methyl-5-(4-[2-sulfonylethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate

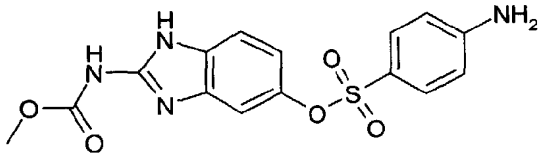
[0052]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 2-aminoethanesulfonic acid with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.6 min., mass spectrum: 471.19, $[M+H]^+$; 941.41, $[2M+H]^+$).

Example 16 : Preparation of Methyl-5-(4-aminophenylsulfonyloxy) benzimidazole-2-carbamate

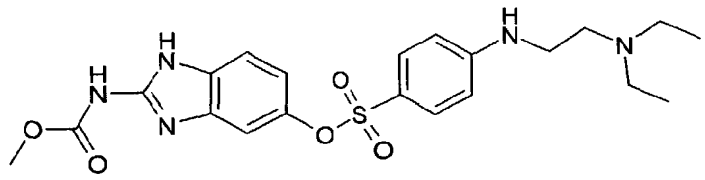
[0053]



In a similar manner to examples 1 to 4, title compound was obtained by reacting ammonia with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.9 min., mass spectrum: 363.19, $[M+H]^+$).

Example 17 : Preparation of Methyl-5-(4-[2-diethylaminoethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate

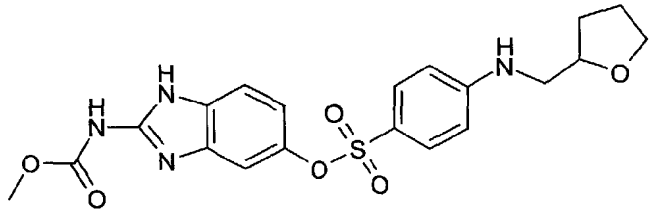
[0054]



In a similar manner to examples 1 to 4, title compound was obtained by reacting 2-diethylaminoethylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 2.6 min., mass spectrum: 462.34, $[M+H]^+$; 923.65, $[2M+H]^+$; 430.30, fragmentation of carbamate: loss of methanol).

Example 18 : Preparation of Methyl-5-(4-[1-tetrathydrofurylmethyl] aminophenylsulfonyloxy) benzimidazole-2-carbamate

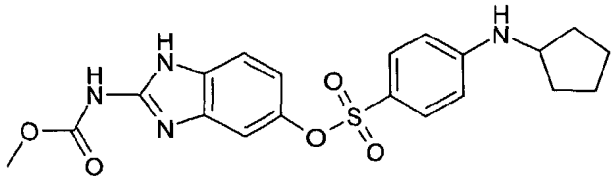
[0055]



In a similar manner to examples 1 to 4, title compound was obtained by reacting tetrahydrofurfurylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.2 min., mass spectrum: 447.24, $[M+H]^+$).

Example 19 : Preparation of Methyl-5-(4-cyclopentylaminophenylsulfonyloxy) benzimidazole-2-carbamate

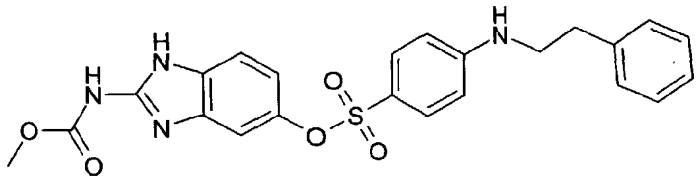
[0056]



In a similar manner to examples 1 to 4, title compound was obtained by reacting cyclopentylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.6 min., mass spectrum: 431.29, $[M+H]^+$).

Example 20 : Preparation of Methyl-5-(4-[2-phenylethyl]aminophenylsulfonyloxy) benzimidazole-2-carbamate

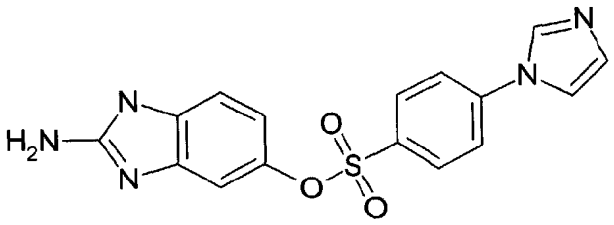
[0057]



In a similar manner to examples 1 to 4, title compound was obtained by reacting phenethylamine with 2-amino-5-(4-fluorophenylsulfonyloxy)nitrobenzene at step 1 of the procedure described above and using nitro group reduction method A. (LC/MS analysis: retention time = 3.6 min., mass spectrum: 467.26, $[M+H]^+$).

Example 21 : Preparation of N-5-(4-[1-imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl : an intermediate for amide product synthesis

[0058]



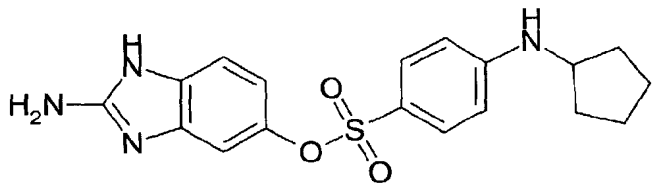
For step 1 and 2, intermediate of title compound is obtained in similar manner to step 1 end 2 of example 4.

[0059] *step 3* : 8 g of step 2 compound were taken up in 128 ml methanol and 21.6 ml acetic acid in a 250 ml round bottom flask. Mixture was heated to reflux and 9.13 g of 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea was added. Reaction mixture was heated to reflux with stirring for 4 hours. Solid was obtained by cooling to 0°C for one hour and washed with ethyl acetate, triturated and dried on a glass frit yielding 7.55 g compound.

[0060] *step 4 :* Compound of step 3 was taken up in 80 ml dichloromethane and 40 ml trifluoroacetic acid. Reaction mixture was stirring for 4 hours at room temperature. Solvents were evaporated under reduced pressure. Concentrated filtrate was taken in 75 ml water. 50 ml of sodium carbonate aqueous solution (10 % w/w). Precipitate obtained was washed with dichloromethane and dried on a glass frit yielding 5.3 g title compound.

Example 22 : Preparation of N-5-(4-[1-imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl: an intermediate for amide product synthesis

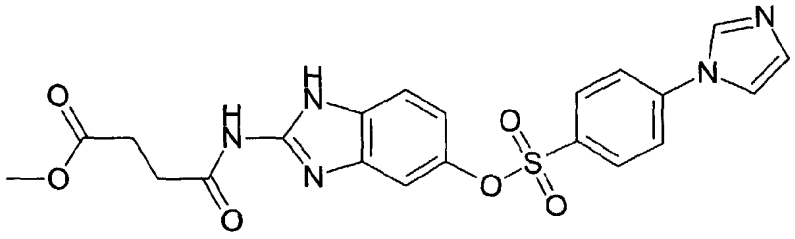
[0061]



For step 1 and 2, intermediate of thitle compound is obtained in similar manner to step 1 end 2 of example 19.
For step 3 and 4, title compound is obtained in similar manner to example 21.

Example 23 : Preparation of N-[5-(4-[1-imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]succinamic acid-methyl-ester.

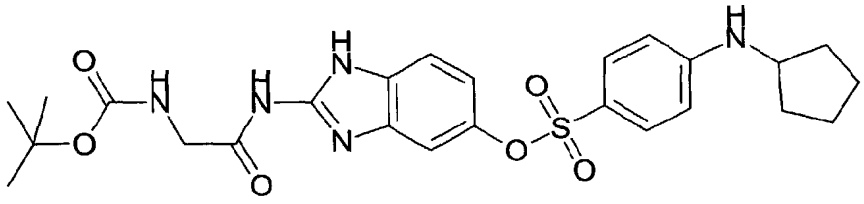
[0062]



step 1 : 8.9 mg of succinamic acid methylester, 25 mg of 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 12 µl diisopropylethylamine were taken up in 0.4 ml dimethylformamide. Reaction mixture was stirred at room temperature for one hour and N-5-(4-[1-imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl was added in 0.2 ml dimethylformamide. Reaction mixture was then stirred at room temperature for 24 hours. Solvent was evaporated in a Jouan model RC 10.10 centrifuge evaporator and title compound was solubilised in 0.5 ml dimethylsulfoxide for LCMS trigged purification yielding 3.9 mg of N-[5-(4-[1-imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]-succinamic-acid-methylester. (LC/MS analysis: retention time = 2.70 min., mass spectrum: 470.34, $[M+H]^+$).

Example 24 : Preparation of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl] *tert*-butoxycarbonyl glycine amide

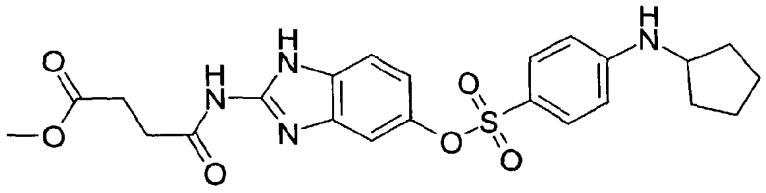
[0063]



step 1 : 11.3 mg of N-(*tert*-butoxycarbonyl)glycine, 25 mg HBTU and 12 µl diisopropylethylamine were taken up in 0.4 ml dimethylformamide. Reaction mixture was stirred at room temperature for one hour and N-5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl was added in 0.2 ml dimethylformamide. Reaction mixture was then stirred at room temperature for 24 hours. Solvent was evaporated in a Jouan model RC 10.10 centrifuge evaporator and title compound was solubilised in 0.5 ml dimethylsulfoxide for LCMS trigged purification yielding 2.4 mg of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-*tert*-butoxycarbonylglycineamid. (LC/MS analysis: retention time = 3.87 min., mass spectrum: 530.38, $[M+H]^+$).

Example 25 : Preparation of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-succinamic acid methyl-ester.

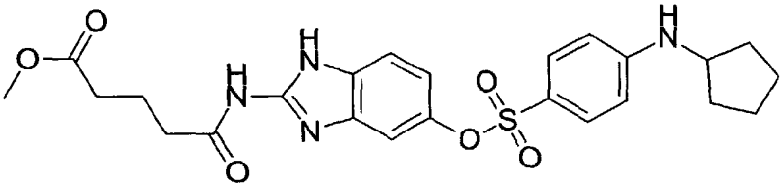
[0064]



In a similar manner to example 24, title compound was obtained by reacting succinamic acid methyl ester with N-5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl. (LC/MS analysis: retention time = 3.72 min., mass spectrum: 487.34, $[M+H]^+$).

Example 26 : Preparation of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-butyric acid methyl-ester.

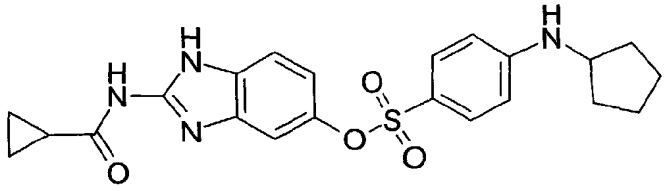
[0065]



In a similar manner to example 24, title compound was obtained by reacting butyric acid methylester with N-5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl. (LC/MS analysis: retention time = 3.75 min., mass spectrum: 501.36, $[M+H]^+$).

Example 27 : Preparation of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-cyclopropanecarbonylamide

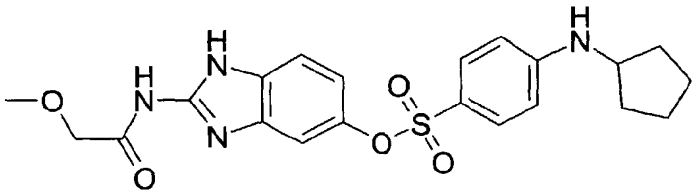
[0066]



In a similar manner to example 24, title compound was obtained by reacting cyclopropane carboxylic acid with N-5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl. (LC/MS analysis: retention time = 3.76 min., mass spectrum: 441.36, $[M+H]^+$).

Example 28 : Preparation of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-methoxyaceticamide

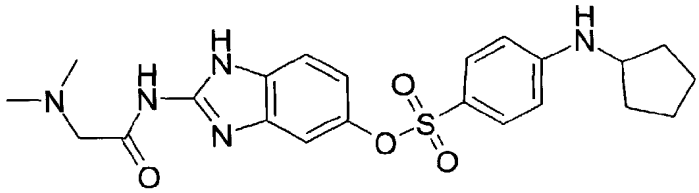
[0067]



In a similar manner to example 24, title compound was obtained by reacting methoxyaceticacid with N-5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl. (LC/MS analysis: retention time = 3.66 min., mass spectrum: 445.34, $[M+H]^+$).

Example 29 : Preparation of N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-dimethylamino-acetylamide

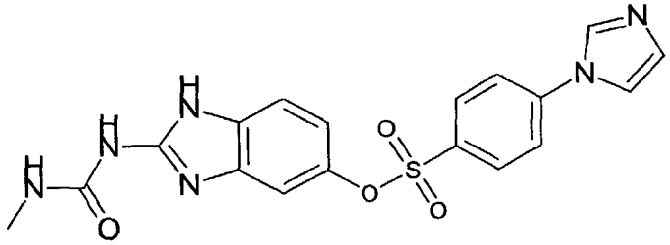
[0068]



In a similar manner to example 24, title compound was obtained by reacting N,N-dimethylglycine with N-5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl. (LC/MS analysis: retention time = 3.36 min., mass spectrum: 458.36, $[M+H]^+$).

Example 30 : N-[5-(4-[imidazolyl]-phenylsulfonyloxy)- 1H-benzimidazole-2-yl]-methylurea

[0069]

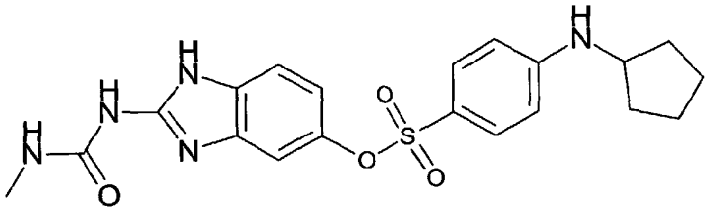


10 mg of Methyl-5-(4-[imidazolyl]-phenylsulfoxy)benzimidazole-2-carbamate (example 4) were combined with 50 µl methylamine (2,0 M in tetrahydrofuran) and 5 µl 1,8-Diazabicyclo[5.4.0]undec-7-ene in 2 ml N-methylpyrrolidone/ tetrahydrofuran (1/1). In a 24 well inox plate for high pressure reaction. The reaction mixture was under a 10 Bars argon pressure and then heated to 80°C for 4 hours, and then cooled at room temperature. Compounds were put in an assay tube and tetrahydrofuran was evaporated under reduce pressure and compound in N-methylpyrrolidone were directly

purified by preparative LCMS in conditions described above. After purification, solution were dry-concentrated in a JOUAN RC1010 evaporator. (LC/MS analysis: retention time = 2.23 min., mass spectrum: 413.23, $[M+H]^+$).

Example 31 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-methylurea

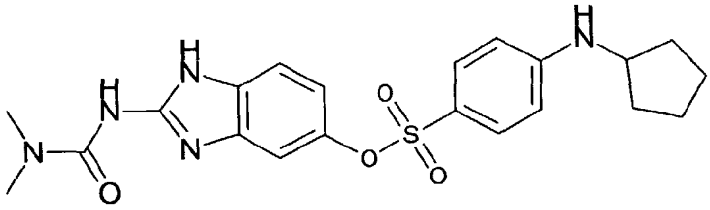
[0070]



In a similar manner to example 30, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with methylamine (2,0 M in tetrahydrofuran). (LC/MS analysis: retention time = 3.30 min., mass spectrum: 430.27, $[M+H]^+$).

Example 32 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-dimethylurea

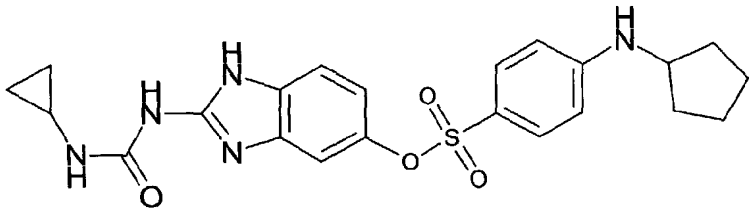
[0071]



Title compound was obtained by reacting 10 mg of Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 50 µl dimethylamine (2,0 M in tetrahydrofuran) and 5 µl 1,8-Diazabicyclo[5.4.0] undec-7-ene in 2 ml dimethylformamide. In a 24 well inox plate for high pressure reaction. The reaction mixture was under a 10 Bars argon pressure and then heated to 80°C for 4 hours, and then cooled at room temperature. Compounds were put in an assay tube and dimethylformamide was dry concentrate evaporated in a JOUAN RC1010 evaporator. Coumpound was diluted in 0.5 ml dimethylsulfoxide for LC/MS trigged purification yielding 9 mg expected (LC/MS analysis: retention time = 3.35 min., mass spectrum: 444.29, $[M+H]^+$).

Example 33 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-cyclopropylurea

[0072]

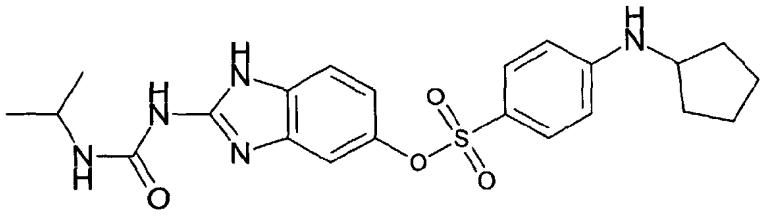


10 mg of Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) were combined with 25 µl cyclopropylamine and 10 µl 1,8-Diazabicyclo[5.4.0]undec-7-ene in 2 ml N-methylpyrrolidone/tetrahydrofuran (0.8/1.2). In a 24 well inox plate for high pressure reaction. The reaction mixture was under a 10 Bars argon pressure

and then heated to 60°C for 40 hours, and then cooled at room temperature. Compounds were put in an assay tube, tetrahydrofuran was evaporated under reduce pressure and compound in N-methylpyrrolidone were directly purified by LC/MS trigged purification yielding 8.7 mg title compound. (LC/MS analysis: retention time = 3.66 min., mass spectrum: 456.36, $[M+H]^+$).

Example 34 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-isopropylamineurea

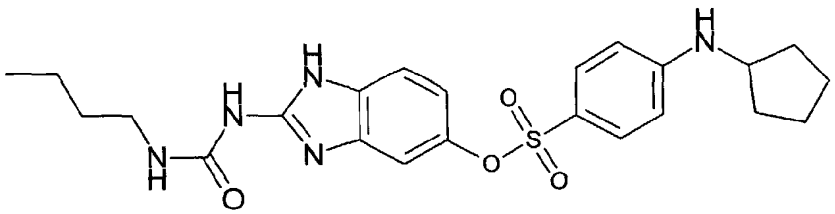
[0073]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with isopropylamine. (LC/MS analysis: retention time = 3.78 min., mass spectrum: 458.36, $[M+H]^+$).

Example 35 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-butylamineurea

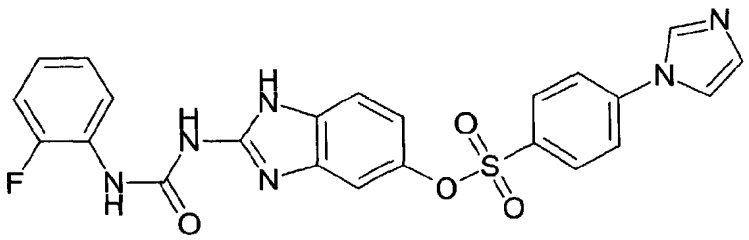
[0074]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with butylamine. (LC/MS analysis: retention time = 3.90 min., mass spectrum: 472.39, $[M+H]^+$).

Example 36 : N-[5-(4-[imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]2-fluoro-anilineurea

[0075]

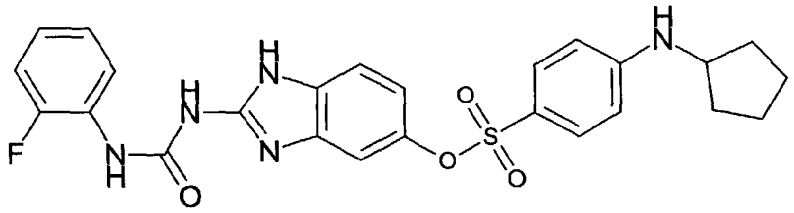


In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-[imidazolyl]-phenylsulfoxy)

benzimidazole-2-carbamate (example 4) with 2-fluoro-aniline. (LC/MS analysis: retention time = 3.03 min., mass spectrum: 493.28, $[M+H]^+$).

Example 37 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl] 2-fluoro-anilineurea

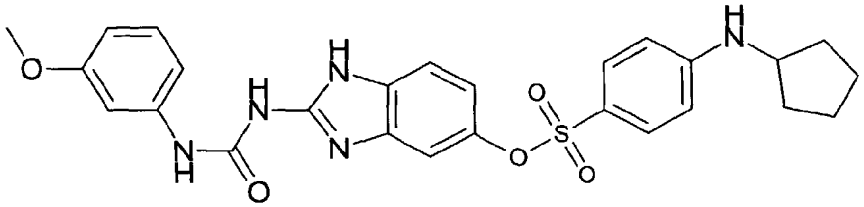
[0076]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 2-fluoro-aniline. (LC/MS analysis: retention time = 3.99 min., mass spectrum: 510.32, $[M+H]^+$).

Example 38 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl] *m*-anisidineurea

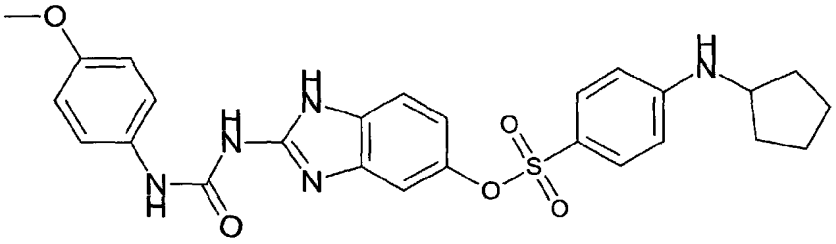
[0077]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with *m*-anisidine. (LC/MS analysis: retention time = 4.02 min., mass spectrum: 522.33, $[M+H]^+$).

Example 39 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-*p*-anisidineurea

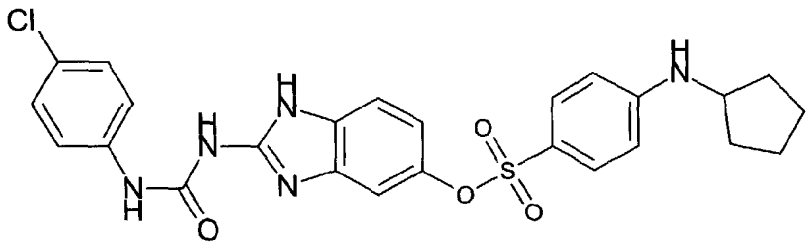
[0078]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with *p*-anisidine. (LC/MS analysis: retention time = 3.97 min., mass spectrum: 522.34, $[M+H]^+$).

Example 40 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-4-chloroanilineurea

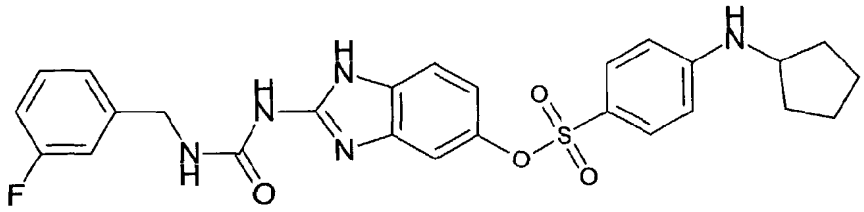
[0079]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 4-chloroaniline. (LC/MS analysis: retention time = 4.20 min., mass spectrum: 526.28, $[M+H]^+$).

Example 41 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-3-fluoro-anilineurea

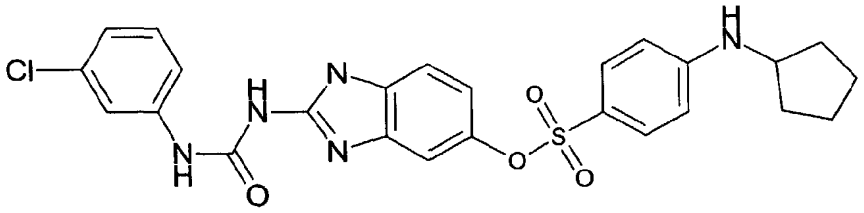
[0080]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 3-fluoro-aniline. (LC/MS analysis: retention time = 3.96 min., mass spectrum: 524.33, $[M+H]^+$).

Example 42 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-3-chloroanilineurea

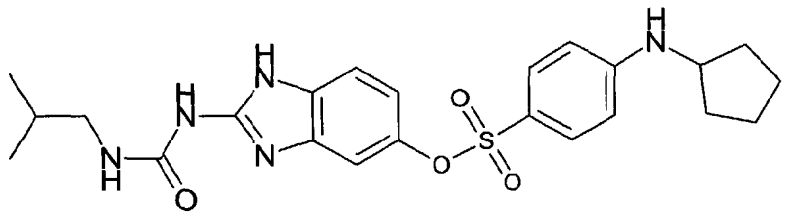
[0081]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 3-chloroaniline. (LC/MS analysis: retention time = 4.21 min., mass spectrum: 526.28, $[M+H]^+$).

Example 43 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-isobutylamineurea

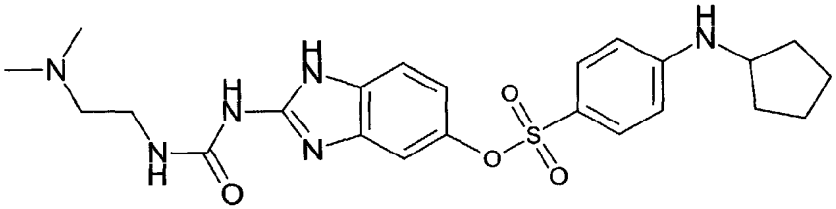
[0082]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with isobutylamine. (LC/MS analysis: retention time = 3.88 min., mass spectrum: 472.38, $[M+H]^+$).

Example 44 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-N,N-dimethylethylenediamineurea

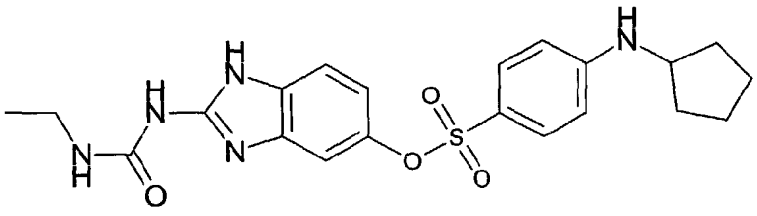
[0083]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with N,N-dimethylethylenediamine. (LC/MS analysis: retention time = 3.22 min., mass spectrum: 487.38, $[M+H]^+$).

Example 45 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-ethylamineurea

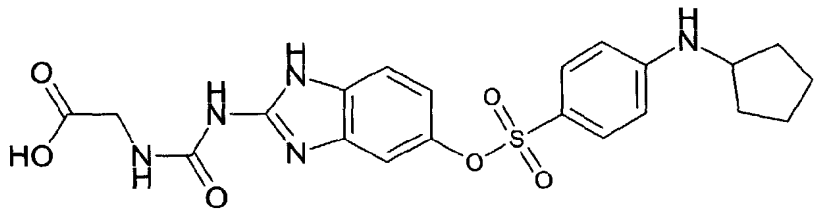
[0084]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with ethylamine (33 % in water). (LC/MS analysis: retention time = 3.64 min., mass spectrum: 444.35, $[M+H]^+$).

Example 46 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-glycineurea

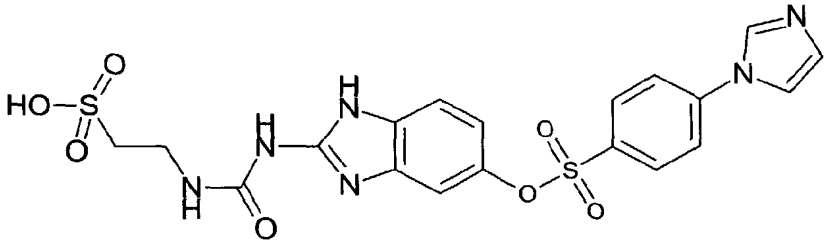
[0085]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with glycine. (LC/MS analysis: retention time = 3.48 min., mass spectrum: 474.31, $[M+H]^+$).

Example 47 : N-[5-(4-[imidazolyl]-phenylsulfonyloxy)-1H-benzimidazole-2-yl]-2-aminoethanesulfonic acidurea

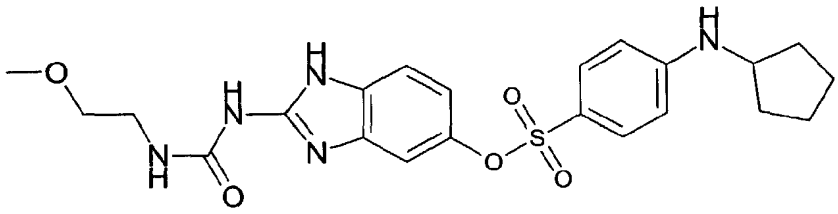
[0086]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-[imidazolyl]-phenylsulfoxy) benzimidazole-2-carbamate (example 4) with 2-aminoethanesulfonic acid. (LC/MS analysis : retention time = 2.40 min., mass spectrum: 507.21, $[M+H]^+$).

Example 48 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl] -2-methoxyethylamineurea

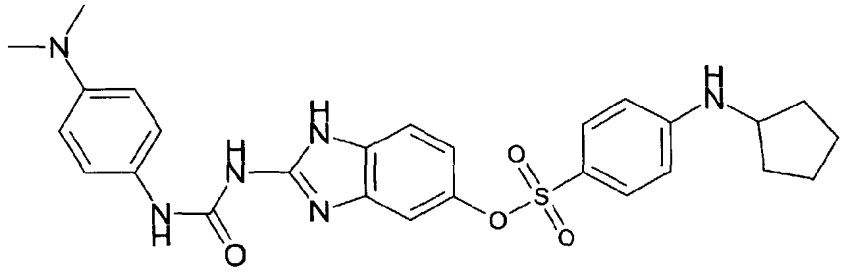
[0087]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 2-methoxyethylamine. (LC/MS analysis: retention time = 3.60 min., mass spectrum: 474.34, $[M+H]^+$).

Example 49 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-N,N-dimethyl-1,4-phenylenediamineurea

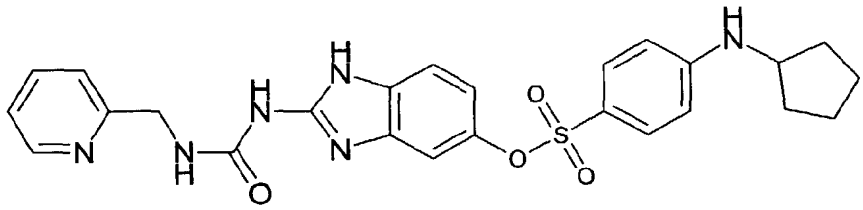
[0088]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with N,N-dimethyl-1,4-phenylenediamine. (LC/MS analysis: retention time = 3.42 min., mass spectrum: 535.34, $[M+H]^+$).

Example 50 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-2-aminomethylpyridineurea

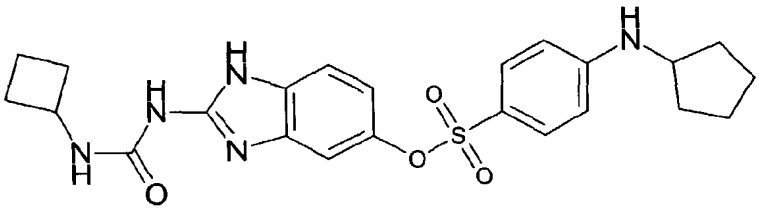
[0089]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 2-aminomethylpyridine. (LC/MS analysis: retention time = 3.30 min., mass spectrum: 507.33, $[M+H]^+$).

Example 51 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)- 1H-benzimidazole-2-yl]-cyclobutylamineurea

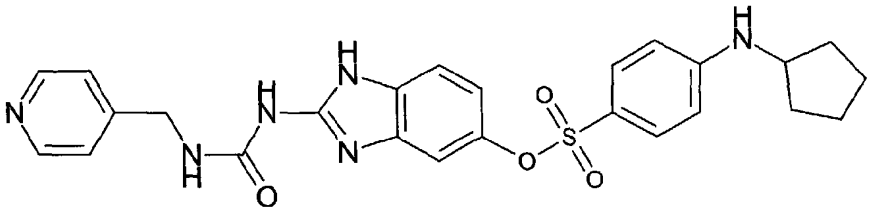
[0090]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with cyclobutylamine. (LC/MS analysis: retention time = 3.84 min., mass spectrum: 470.36, $[M+H]^+$).

Example 52 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]-4-(aminomethyl)pyridineurea

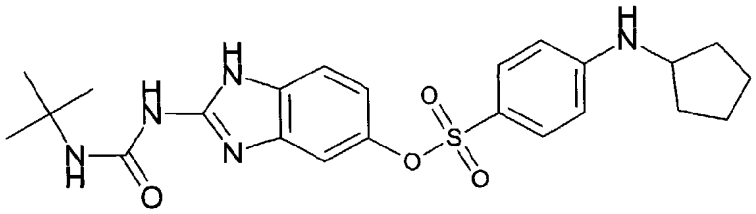
[0091]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with 4-(aminomethyl)pyridine. (LC/MS analysis: retention time = 3.24 min., mass spectrum: 507.33, $[M+H]^+$).

Example 53 : N-[5-(4-cyclopentylaminophenylsulfonyloxy)-1H-benzimidazole-2-yl]- *tert*-butylamineurea

[0092]



In a similar manner to example 33, title compound was obtained by reacting Methyl-5-(4-cyclopentylaminophenylsulfonyloxy)benzimidazole-2-carbamate (example 19) with *tert*-butylamine. (LC/MS analysis: retention time = 3.93 min., mass spectrum: 472.36, $[M+H]^+$).

Biological tests

[0093] The experiments described in this report were designed to evaluate the cytotoxicity of *"in vitro"* Cdk4 inhibitors in comparison with Staurosporine, a non-specific Serine-Threonine kinase inhibitor.

[0094] Stock solutions of compounds were made in DMSO at 10 mM and stored at -20°C. Subsequent dilutions were made in 28 % DMSO and used to add 3 µl of the drugs at varied concentrations to the HeLa cells.

[0095] All cell lines were cultured at 37°C in a humidified atmosphere containing 5 % CO_2. HeLa human epithelial cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were grown as monolayers in Dubelcco's Modified Eagle Medium containing 2 mM L-glutamine, 200 I.U./ml penicillin, 200 µg/ml streptomycin, and supplemented with 10 % (v/v) heat inactivated foetal calf serum. Cells were transferred twice a week at 10^5 cells/ml in 75 cm^2 flasks after trypsinisation. Different flasks were done to prepare two preparations the day of experiment.

Cell growth inhibition

[0096] Cells in exponential phase of growth were trypsinised and resuspended in their culture medium at 2.5 10^4 cells/ml, in two independent preparations. Cell suspension was distributed in 96 well Cytostar microplates (Amersham) (0.2 ml/well, 5000 cells). Hela cells were coated for 4 hours at 37°C. [^{14}C]-thymidine (0.1 µCi/well) and ten final concentrations of molecules (3 µl) ranging from 20 to 0.03 µM were then added. The uptake of [^{14}C]-thymidine was measured 48h after the labelling had been started using a Microbeta Trilux counter (Wallac).

[0097] Staurosporine, the reference compound, was evaluated using the same procedure.

[0098] CPM measured 48 hours after the test substance had been added to the media, were compared to those

obtained with 0.4 % final DMSO, in the control wells.

[0099] IC_{50}, obtained from a dose response curve of 10 concentrations in duplicate is the concentration of drug wich diminishes half the specific signal. It is determined by non-linear regression analysis and calculated as a concentration at middle of curve.

[0100] IC_{50} values result from 2 independent experiments for all tested molecules.

CDK4/CyclinD1 Flashplate Assay: 96-well format

[0101] This is a CDK4/CyclinD1 kinase assay in a 96-well Streptavidin-coated Flashplate with a biotinylated-Rb peptide substrate.

[0102] Each point is tested in duplicate

[0103] Biotinylated-Rb: Biotin-RPPTLSPIPHIPRSPYKFPSSPLR

Kinase Buffer:

[0104]

HEPES, pH 8	50 mM
$MgCl_2$ $6H_2O$, pH 7	10 mM
DTT	1 mM

1. Prepare substrate: 1 mg/ml solution made fresh in PBS.

2. Add 100 µg per well to the Flashplate.

3. Incubate for 2 hours at RT.

4. From 10 mM inhibitor stocks in DMSO, make 1 mM, 300 µM, 100 µM, 30 µM and 10 µM series of dilution in DMSO.

5. Wash the Flashplate 3 times with 300 µl PBS to remove unbound peptide substrate.

6. Add the CDK4/CyclinD1 kinase: 70 ng per well, in a volume of 90 µl in kinase buffer (except for "no enzyme" control wells).

7. Add 1 µl per well of inhibitor to test 10 µM, 3 µM, 1 µM, 0.3 µM and 0.1 µM in final concentration per 100 µl in each well.

8. Shake gently the Flashplate 1 minute.

9. Incubate 30 minutes on wet ice.

10. Initiate the reaction with 10 µl kinase buffer containing 1 µM final cold ATP and 1 µCi final ^{33}P-ATP per well.

11. Shake gently the Flashplate 1 minute.

12. Incubate 45 minutes at RT (no shaking).

13. Wash the Flashplate 3 times with 300 µl PBS

14. Count to detect the incorporation of ^{33}P-ATP by the kinase to the Rb phosphorylation site.

Example N°	IC50 CDK4 n(µM)
1	1,43 / 1,65
2	2,35 / 1,58

(continued)

Example N°	IC50 CDK4 n(μM)
3	1,27 / 0,48
4	1 / 3
5	1,67 / 1,63
6	0,7 / 0,7
7	0,77 / 0,17
8	1,3 / 0,88
9	2,23 / 1,78
10	0,29 / 0,13
11	0,98/0,36/0,46
12	14% inhibition at 10 μM
13	0,31 / 0,48
14	0,64 / 0,3
15	<0.3 / 0,32
16	0,28 / 0,47
17	5,47 / 7,16
18	1,44 / 1,22
19	1,45 / 0,67
20	>3

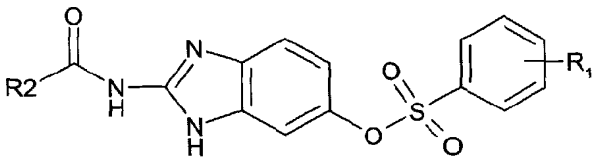
Example N°	% Inhib. (10 μM) Cdk4	IC50 (μM) Cdk4
31	nd	0,55 / 0,51
32	nd	1,81 3,13
30	nd	1,63 / 1,9
23	84 / 83	
24	92 / 94	
25	100 / 100	
26	92 / 90	
27	88 / 89	
28	87 / 88	
29	91 / 92	
33	95 / 96	
34	87 / 92	
35	89 / 84	
36	89 / 92	
37	95 / 95	

(continued)

Example N°	% Inhib. (10 μM) Cdk4	IC50 (μM) Cdk4
38	84 / 85	
39	89 / 89	
40	89 / 89	
41	85 / 83	
42	85 / 83	
43	85 / 88	
44	93 / 95	
45	94 / 96	
46	99 / 100	
47	88 / 88	
48	97 / 96	
49	88 / 88	
50	97 / 97	
51	90 / 91	
52	91 / 91	
53	82 / 83	

Claims

1. Compounds of formula (I)



(I)

- wherein R_1 is selected from the group consisting of
  - 4 NH_2
  - 4 NH alkyl or cycloalkyl eventually substituted with an an acyl, an acylderivative, an hydroxy, an amino, alkoxy, heterocyclyl or aryl group
  - 4 N imidazolyl
  - 3 SO_2 Me
- wherein R2 is selected from the group consisting of
  - alkyl eventually substituted by amino, acid, acid derivative, alkoxy, aryl or OH groups
  - arylalkyl eventually substituted by alkoxy, halogeno, amino, acid or acid derivatives
  - alkoxy eventually substituted by aryl
  - amino, NHR_3, NR_3R_4 wherein R_3 and R_4 are selected independtly from hydrogen, alkyl, alkylaryl, aryl or

together form an alkylen chain

or a pharmaceutically acceptable salt.

2. Compounds according to claim 1 wherein the aryl substituents are substituted with one or more groups selected from halogen, alkoxy, alkyl, amino, nitro.

3. Compounds according to claim 1 wherein the alkyl substituents are substituted with one or more amino, acyl, acyl derivatives, aminoacyl derivatives, alkoxy, arylalkyl or aryl groups.

4. Compounds according to claim 1 wherein the alkoxy substituents are substituted with one or more amino, acyl, acyl derivatives, alkyl, arylalkyl or aryl groups

5. Compounds according to claim 1 wherein the acyl groups or acyl derivatives groups include carboxylic acids and sulfonic acids or derivatives.

6. Compounds according to claim 5 wherein the acid derivatives include alkyl esters.

7. Compounds according to claim 1 wherein the alkyl chains are linear, ramified or cyclic and contain 1 to 7 carbon atoms.

8. Compounds according to claim 1 wherein the alkoxy chains are linear, ramified or cyclic and contain 1 to 4 carbon atoms.

9. Compounds according to claim 1 wherein the aryl groups include phenyl group, heterocyclyl groups containing one or two heteroatoms choosen from S, N.

10. Compounds according to claim 7 wherein the alkyl chain contain 1 to 4 carbon atoms and the cycloalkyl chain contains 3 to 5 carbon atoms.

11. Compounds according to claim 8 whrein the alkoxy group is a methoxy group.

12. Compounds according to claim 1 wherein in formula (I) R_2 is an amino substituent choosen among a monoalkylamino or a monoarylamino substituent

13. Pharmaceutical composition, which comprises a therapeutically effective amount of a compound of formula (I) in combination with a pharmaceutically acceptable carrier.

14. Use of compounds of formula (I) for the preparation of a drug.

15. Use of compounds of formula (I) for the preparation of a drug for treating cancer deseases.

16. Use of the compound of formula (I) as CDK4 inhibitors.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 40 2460

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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Y	LACEY E ET AL: "ACTIVITY OF BENZIMIDAZOLE CARBAMATES AGAINST L1210 MOUSSE LEUKEMIA CELLS: CORRELATION WITH IN VITRO TUBULIN POLYMERIZATION ASSAY" BIOCHEMICAL PHARMACOLOGY, PERGAMON, OXFORD, GB, vol. 34, no. 19, 1985, pages 3603-3605, XP002056333 ISSN: 0006-2952 * page 3604; example 13; table 1 *	1-16	
A	KRUSE L I ET AL: "Synthesis, tubulin binding, antineoplastic evaluation, and structure-activity relationship of oncodazole analogues" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 32, no. 2, February 1989 (1989-02), pages 409-417, XP002142971 ISSN: 0022-2623 * page 413, column 1, paragraphs 3,4 *	1-16	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07D

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	26 February 2002	Timmermans, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82